2018-04

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10.1016/j.orggeochem.2018.01.008
Organic Geochemistry
Elsevier BV

Sterol identification in floating Arctic sea ice algal aggregates and the Antarctic sea ice diatom *Berkeleya adeliensis*.

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ABSTRACT

A number of common sterols were identified in sea ice diatoms from the Arctic and the Antarctic. The main sterols in floating sea ice algal aggregates collected from Resolute Passage (Canadian Arctic) and Nansen Basin (North Svalbard) in 2012 were 22E-dehydrocholesterol, cholesterol, epi-brassicasterol, 24-methylenecholesterol and 24-ethylcholesterol, although the distribution varied between the two locations, likely reflecting compositional differences in

diatom taxa. The three major sterols in cells of *Berkeleya adeliensis* picked from a melted sea ice core collected from Ryder Bay in the Antarctic Peninsula in 2014, were 24-ethylcholesterol, cholesterol and 22E-dehydrocholesterol. We suggest that certain sea ice diatoms may thus contribute to the sedimentary budget of common sterols in seasonally sea ice-covered locations following ice melt.

1. Introduction

Sterols are amongst the most common lipid constituents of diatoms (e.g. Volkman, 1986; Rampen et al., 2010). In recent years, the analysis of a number of sterols including 24-methylcholesta-5,22E-dien-3β-ol (brassicasterol or epi-brassicasterol) has been used to provide information regarding surface ocean settings in palaeo Arctic sea ice reconstructions based on the organic geochemical sea ice proxy IP_{25} (Belt et al., 2007; Belt and Muller, 2013). In particular, the occurrence of, respectively, relatively high or low sedimentary concentrations of certain sterols has been employed as a means of distinguishing between permanently open-water versus perennially ice-covered regimes when IP_{25} is typically absent (or very low in concentration), as first demonstrated by Müller et al. (2009). However, such interpretations are likely made more complicated by the fact that many sterols also have non-marine sources (Huang and Meinschein, 1976; Volkman, 1986), such that the possibility of, for example, terrestrial input via fluvial discharge may complicate the marine sedimentary signal. Further, for regions of seasonal sea ice cover, there is the additional likely biosynthesis of sterols by common sympagic biota, especially as sterols appear to be common to

all diatoms, the most abundant phototrophs found in sea ice. Consistent with this, a number of sterols have previously been reported in both Arctic and Antarctic sea ice samples containing high diatom content (Nichols et al., 1989, 1993; Brown et al., 2011; Belt et al., 2013). However, since some sea ice also contains material entrained from the water column during freeze-up (e.g. suspended sediment), a question remains as to the true origin of certain sterols in previously analysed sea ice samples. On the other hand, the magnitude of sterol concentrations in sea ice reported in previous studies (typically tens to hundreds of ng sterol per ml sea ice melt; Brown et al., 2011; Belt et al., 2013) likely precludes allochthonous sources as major contributors, albeit in the relatively few studies reported thus far. To help clarify matters, here we analysed the sterol content in (i) floating sea ice diatom aggregates from two different regions of the Arctic, including some from which the sources of the sea ice diatom biomarker IP_{25} had previously been identified (Brown et al., 2014) and (ii) picked cells of *Berkeleya adeliensis*, a well-known constituent of Antarctic sympagic diatom communities, and a recently identified source of $IPSO_{25}$, the proposed counterpart sea ice proxy to IP_{25} in the Southern Ocean (Belt et al., 2016). Our data confirm the biosynthesis of certain sterols by sympagic diatoms, as proposed in previous studies (Nichols et al., 1989, 1993; Brown et al., 2011; Belt et al., 2013).

2. Methods

Floating sea ice algal aggregates (four samples from Resolute Passage, Arctic Canada and one from the Nansen Basin north of Nordaustlandet,

Svalbard (both 2012)) and sea ice cores (Ryder Bay, Antarctic Peninsula (2014)) were obtained as described previously (Assmy et al., 2013; Brown et al., 2014; Belt et al., 2016). Floating algal aggregates were washed with de-ionised water to remove salts and then freeze-dried. Individual cells of *B. adeliensis* were picked from a partially thawed section of sea ice by hand using a modified pipette. In all cases, the resulting sympagic diatom samples were extracted with hexane (1 ml, ultrasonication; 5 min) and fractionated using column chromatography (0.5 g SiO_2) to obtain apolar lipids (5 ml hexane) and sterols (5 ml hexane/methyl acetate (4:1 v/v)). Sterol fractions were derivatised using N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA; 50 µl; 70°C; 60 min) and analysed using gas chromatography–mass spectrometry (GC–MS) in total ion current (TIC) or selected ion monitoring (SIM) mode using an Agilent 7890a Series II gas chromatograph, fitted with a 30 m fused silica HP_{5ms} column (0.25 mm i.d., 0.25 µm film) coupled to a 5975c Series Mass Selective Detector (MSD). Individual sterols were identified by comparison of the mass spectra of their TMS ethers with published data (e.g. Volkman, 1986). 24-methylcholest-5,22E-dien-3β-ol was assumed to be epi-brassicasterol since this isomer is found in diatoms (e.g. Volkman, 1986; Volkman et al., 1998).

3. Results and Discussion

3.1 Sterols in Arctic sea ice diatoms

The most abundant sterols in the four floating ice algal aggregate samples from Resolute Passage in the Canadian Arctic could be readily identified by comparison of their characteristic mass spectra with those reported previously.

Some other components were either too low in concentration to permit reliable identification or had mass spectra that did not match those of known sterol-TMS ethers. For each of the four samples, the principal identifiable sterols were cholesta-5,22E-dien-3β-ol (22E-dehydrocholesterol), cholest-5-en-3β-ol (cholesterol), 24-methylcholest-5,22E-dien-3β-ol (epi-brassicasterol), 24-methylcholest-5,24(28)-dien-3β-ol (24-methylenecholesterol), 24-methylcholest-5-en-3β-ol (methylcholesterol) and 24-ethylcholest-5-en-3β-ol (24-ethylcholesterol), with epi-brassicasterol, 24-methylenecholesterol and 24-ethylcholesterol as the major constituents (Fig. 1a; Table 1). These findings are consistent with those of Belt et al. (2013) who reported very similar sterol compositions in filtered sea ice samples collected from the same region and season in 2011 and 2012. A similar series of sterols was also present in the floating ice algal sample collected from Nansen Basin, northern Svalbard, although with a slightly different distribution compared to those found for the samples from Resolute Passage (Fig. 1b; Table 1), probably due to changes in diatom taxa content. Indeed, the ice-associated pennate diatoms *Navicula pelagica*, *Nitzschia frigida* and *Pauliella taeniata* were the major taxa in the aggregates from Resolute Passage (Brown et al., 2014), while *N. pelagica*, *Hantzschia weyprechtii*, *Entomoneis paludosa* and *Cylindrotheca closterium* were the most abundant species in the sample from the Nansen Basin (Assmy et al., 2013).

3.2 Sterols in the Antarctic sympagic diatom B. adeliensis

The main sterols in the picked cells of *B. adeliensis* were 22-dehydrocholesterol, cholesterol and 24-ethylcholesterol (Fig. 1c; Table 1). Previously, Nichols et al. (1989, 1993) identified the same three sterols in filtered

sea ice samples from McMurdo Sound, Antarctica, containing mixed diatom communities, in which *Berkeleya spp.* were also identified. In our picked cells of *B. adeliensis*, trace amounts of epi-brassicasterol and 24-methylcholesterol could also be identified by GC–MS in SIM mode. These sterols were more prominent in some of the mixed assemblages of sea ice diatoms analysed by Nichols et al. (1989, 1993), presumably reflecting the changes in diatom composition in those samples.

Finally, since the deposition of Arctic and Antarctic sea ice diatoms in underlying sediments is well known from micropalaentological (Armand et al., 2017) and biomarker studies (e.g. via the detection of IP_{25} and $IPSO_{25}$; Belt et al., 2007, 2016; Belt and Müller, 2013), it follows that they likely also contribute to the sedimentary sterol budget in seasonally sea ice-covered locations.

4. Conclusions

The results presented herein confirm the production of certain common sterols by sympagic algae in both the Arctic and Antarctic, as proposed in some previous studies. Of course, these findings do not preclude the possibility of some sterol entrainment (e.g. from sediments) in sea ice from other locations, which subsequently become deposited in underlying sediments following ice melt. Indeed, resolving the relative source contributions of various sterols in marine sediments will likely remain a challenge for such common lipid biomarkers.

5. Acknowledgments

We thank the University of Plymouth for funding. Sample collection (Resolute Passage) was supported through a Natural Sciences and Engineering

Research Council of Canada grant to C.M. P.A. was supported by the Centre of Ice, Climate and Ecosystems at the Norwegian Polar Institute and the Research Council of Norway (project no. 244646). We thank Mairi Fenton for providing the sea ice sample from Ryder Bay. Finally, we acknowledge John Volkman and Andy Revill for providing constructive feedback on the original manuscript.

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198 **Figure legends**
199 Figure 1. Partial GC – MS chromatograms showing the presence of various
200 sterols in Arctic and Antarctic sea ice diatoms: (a) floating sea ice aggregates
201 from Resolute Passage (Canadian Arctic) in 2012 (sample RP-1); (b) floating sea
202 ice aggregates from Nansen Basin (North Svalbard) in 2012; (c) Individual cells
203 of *B. adeliensis* picked from a landfast sea ice core from Ryder Bay (Antarctic
204 Peninsula) in 2014. Sterols are numbered as follows: (1) 22E-dehydrocholesterol;
205 (2) cholesterol; (3) epi-brassicasterol; (4) 24-methylenecholesterol; (5) 24-
206 methylcholesterol; (6) 24-ethylcholesterol.
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209 Table 1. Percentages of sterols in sea ice algal samples. Resolute Passage (RP);

210 Nansen Basin (NB).

211

	Sample					
Sterol	RP-1	RP-2	RP-3	RP-4	NB	*B. adeliensis*
22E-dehydrocholesterol	10	9	11	10	19	7
cholesterol	4	4	5	4	17	40
epi-brassicasterol	30	29	29	32	24	tr
24-methylenecholesterol*	28	26	26	26	15	nd
24-methylcholesterol	tr	tr	tr	tr	tr	tr
24-ethylcholesterol	29	32	29	28	25	53

*includes small amounts of 24-methylcholesterol

tr: trace amounts

nd: not detected

212

213 Figure 1.